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Early detection of phosphorus deficiency stress in cucumber at the cellular level using chlorophyll fluorescence signals

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Abstract: Abiotic stressors contribute to growth restriction and developmental disorders in plants. Early detection of the first signs of changes in plant functioning is very important. The objective of this study was to identify chlorophyll fluorescence parameters that change under phosphorus deficiency stress in cucumber. In this work, a trail to study the early changes caused by phosphorus deficiency in cucumber plants by analysing their photosynthetic performance is presented. Chlorophyll-*a* fluorescence (ChF) parameters were measured every 7 days for a period of 28 days. Measurements were made separately on young and old leaves and on cucumber fruit. Parameters that decreased during the stress were: *p2G*, PI_{abs}, PI_{total}, RE_o/CS_o, and TR_o/CS_o. P deficiency decreased total electron carriers per *RC* (EC_o/RC), yields (TR_o/ABS (F_v/F_m), ET_o/TR_o, RE_o/ET_o, ET_o/ABS and RE_o/ABS), fluxes (RE_o/RC and RE_o/CS_o) and fractional reduction of PSI end electron acceptors, and damaged all photochemical and non-photochemical redox reactions. Principal component analysis revealed a group of ChF parameters that may indicate early phosphorus deficiency in cucumber plants. Our results are used in the discovery of sensitive bioindicators of phosphorus deficiency in cucumber plants. Most JIP test parameters are linked to mathematical equations, so we recommend using of advanced statistical tools, such as principal component analysis, which should be considered very useful for stress identification. It has also been shown to be more effective in multivariate methods compared to univariate statistical methods was demonstrated.

Keywords: abiotic stress, chlorophyll fluorescence, cucumber, JIP test, multi-dimensional statistical analyses, photosynthesis

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INTRODUCTION

Phosphorus (P) is an essential heteroelement in compounds such as adenosine triphosphate (ATP), nicotinamide adenine dinucleotide phosphate (NADPH), nucleic acids, sugars, phosphates, and phospholipids, all of which play important roles in photosynthesis [White, Hammond 2008]. Consequently, even a moderate phosphorus deficiency has significant effects on plant growth and development.

Various methods have been used to assess the nutritional status of plants. Most of them are destructive, time-consuming and expensive, such as proteins and chemical analysis. One of the new promising methods is the non-destructive measurement of chlorophyll-*a* fluorescence [Alexandrov *et al.* 2014; Cetner *et al.* 2020; Horaczek *et al.* 2020; Samborska *et al.* 2018; Sitko *et al.* 2019]. The phenomenon of chlorophyll-*a* fluorescence is used to determine the efficiency of the photosynthetic apparatus, especially the photosystem II (PSII), and the general health of the plant [Baker, Rosenquist 2004]. Measurements of chlorophyll fluorescence parameters provide specific information about the state of the photosynthetic apparatus and damage within chloroplasts [Kalaji *et al.* 2017a]. They also help to estimate the effects of environmental factors on plant growth and yield [Maxwell, Johnson 2000; Urmi *et al.* 2022]. Most of the research conducted emphasises that there are two parameters related to chlorophyll fluorescence, namely the power index (*PI*) and the maximum quantum yield of PSII photochemistry (F_v/F_m) [Force *et al.* 2003; Strasser *et al.* 2000].

Based on the ease of performing chlorophyll-*a* fluorescence measurements, the observed specificity, and the successful prediction model, it appears that OJIP transients can be used as a valuable probe to determine the concentration of bioactive phosphorus in plants [Cetner *et al.* 2020]. This dynamic fluorescence analysis is an excellent tool for developing unique, site-specific phosphorus fertilisation strategies based on timely measurements of the current status of this element in plants, ensuring optimal yields while avoiding excessive phosphorus application. In addition, research indicates that similar detection of deficiencies in other nutrients is also possible [Donnini *et al.* 2003; Frydenvang *et al.* 2015].

Measurement of chlorophyll-*a* fluorescence has been used extensively to study photosynthetic efficiency in various species subjected to macro- and micronutrient deficiencies, such as sugar beets [Belkhodia *et al.* 1998], pears [Morales *et al.* 2000], peaches [Molassiotis *et al.* 2006], and poplars [Solti *et al.* 2008]. Analysis of chlorophyll-*a* fluorescence in pear [Morales *et al.* 2000], tomato [Donnini *et al.* 2003], peach [Molassiotis *et al.* 2006], and pea [Jelali *et al.* 2011] showed a general increase in non-photochemical quenching (qNP) in chlorotic leaves, indicating the activation of photoprotective mechanisms, but not sufficient to prevent photoinhibition, as shown by a significant decrease in maximum PSII photochemistry (F_v/F_m) and photochemical quenching (qp). The actual quantum yield of PSII showed the same behaviour with respect to qp and F_v/F_m.

Phosphorus is involved at various stages of light energy absorption, transfer and conversion within thylakoids membranes (Fig. 1). Recently, even minimal phosphorus deficiency was shown to have a significant effect on electron transfer between PSII and photosystem I (PSI) [Frydenvang *et al.* 2015]. Analysis of chlorophyll-*a* fluorescence (OJIP) transients showed that stage I of the electron transport chain was significantly perturbed by phosphorus deficiency and that the change in fluorescence curvature can be used to quantify the bioactive phosphate pool in plants. However, there are no detailed data on how the

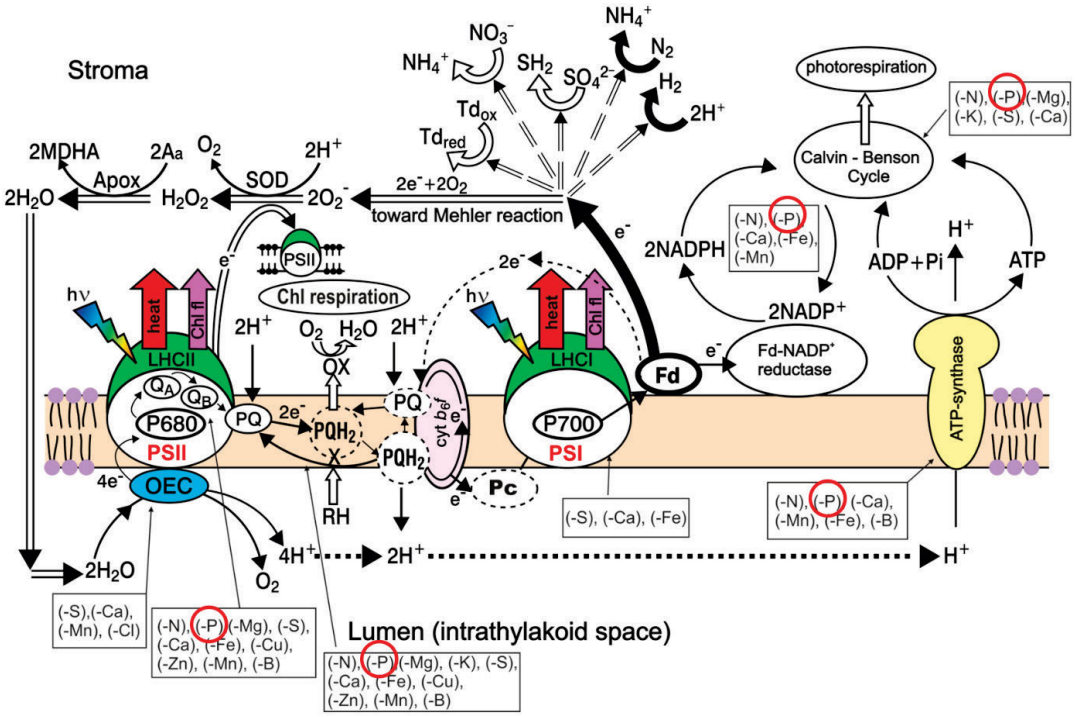

Fig. 1. Contribution of various nutrients (including P; marked with a red circle) at various stages of light energy absorption, transfer and conversion within thylakoids membranes; LHCI = light harvesting complex I; LHCII = light harvesting complex II; source: Kalaji *et al.* [2017a], modified

functioning of the photosystem II changes under phosphorus deficiency stress and whether there is a set of chlorophyll-*a* fluorescence parameters that could be used to confirm this in the case of cucumber plants. In this study, we hypothesised that phosphorus deficiency stress induces specific changes in the photosystem II and therefore can be quickly and non-destructively determined/predicted using chlorophyll-*a* fluorescence measurements.

The objective of this study was to identify chlorophyll fluorescence parameters that change under phosphorus deficiency stress in cucumber. Identification of such parameters may help in early detection of phosphorus deficiency stress. The use of a simple, rapid and non-invasive method such as the chlorophyll-*a* fluorescence measurements may contribute to better plant health, which should lead to higher yields.

MATERIALS AND METHODS

PLANTS GROWTH CONDITIONS AND EXPERIMENT DESIGN

The effects of phosphorus deficiency on the developmental parameters of cucumber and the first symptoms on the plant were studied. The study was carried out at Warsaw University of Life Sciences (SGGW) in the greenhouse (longitude 21°E, latitude 51°15'N) in winter cycle with assimilation light, using the hydroponic method in rockwool substrate from Grodan. The greenhouse cucumber cultivar 'Melen'F1 by Rijk Zwaan was used for the study. Plants were grown on one fruiting shoot. The microclimate parameters in the growing room were on average: temperature 22–25°C/18–22°C day/night, CO_2 800 ppm, relative humidity (*RH*) on average 70%. At low solar radiation the plants were illuminated with Philips LED lamps. Photosynthetic photon flux density (*PPFD*) was maintained at ~320 μmol·m^{-2}·s^{-1} in the cultivation chamber. The nutrient solution for fertigation was based on single-component fertilisers and a multi-component micronutrient fertiliser (superba mikromix by Yara). The pH and electrical conductivity (*EC*) parameters of the nutrient solution averaged 5.7 and 3.2–3.3 mS·cm^{-1}, respectively. The microclimate and fertilisation parameters were controlled by computer. Phosphorus deficiency (–P) was induced in cucumber at the full fruiting stage of the plants.

The experiments were performed on randomly selected plants in three replications of 6 plants each. Until induction of P deficiency in the medium, all plants were fertilised with complete medium (C; Control), which in 1 dm^3 contained: 195 mg N-NO_3, 6 mg N-NH_4, 56 mg P, 264 mg K, 44 mg Mg, 257 mg Ca, 2.5 mg Fe, 0.83 mg Mn, 0.68 mg B, 0.15 mg Cu, 0.14 mg Zn, 0.08 mg Mo. Half of the cucumber plants, 82 days after sowing (DAS) the seeds were started by treating the plants with nutrient solution without phosphorus (–P) and the other components were as in the control. The other half of the plants, the control (C) was treated as before with nutrient solution containing all minerals. This term was designated as term 0.

In each combination, 6 test plants were randomly selected for observation and measurements. The measurements were performed at the following terms: term 0 – day of phosphorus withdrew from cucumber fertigation medium, term 1–7 days of cucumber growth with nutrient solution without phosphorus (–P), term 2–14 days of cucumber growth with nutrient solution without phosphorus (–P), term 3–21 days of cucumber growth with nutrient solution without phosphorus (–P), term 4–28 days of cucumber growth with nutrient solution without phosphorus (–P).

In the test plants, the study was conducted on the 5th fully developed leaf counting from the top of the cucumber plant (young leaf) and on the 10th leaf counting from the top of the plant (old leaf). The results from the 5th and 10th leaves were averaged and given as the mean result per plant. Fruits ripe for harvesting were harvested from the main shoot, every other day.

CHLOROPHYLL FLUORESCENCE MEASUREMENTS

Chlorophyll fluorescence measurements were performed using the HandyPEA fluorimeter. Measurements were made after samples were allowed to adjust to darkness for 30 min. The following measurement protocol was applied on young and old leaves and on fruits, separately: measurement time – 1.0 s, actinic light intensity – 3500 μmol·m^{-2}·s^{-1}, wavelength – 635 ±10 nm. One measurement resulted in the detection of 118 points within 1 s. The JIP test described by Strasser *et al.* [2000] was used to back-calculate the characteristic points of the photoinduced chlorophyll fluorescence transients to specific parameters of the light phase of photosynthesis. All measured and calculated parameters are described in Table 1.

Table 1. Summary of measured and calculated chlorophyll-*a* fluorescence parameters

Fluorescence parameter	Description
t for F_m	time (in ms) to reach maximal fluorescence F_m
Area (A_m)	area above the OJIP curve between F_o and F_m and the F_m asymptote
Area to F_J	area above the OJIP curve between F_o and F_J asymptote
F_o	minimum fluorescence, when all PSII *RCs* are open, fluorescence intensity at 50 μs
$F_m = F_P$	maximum recorded fluorescence at *P*-step
$F_v = F_m - F_o$	maximum variable fluorescence
$F_K = F_{0.3ms}$	fluorescence intensity at *K*-step (0.3 ms)
F_J = 2 ms	fluorescence intensity at the *J*-step (2 ms)
F_I = 30 ms	fluorescence intensity at the *I*-step (30 ms)
F_o/F_m	minimum fluorescence, when all PSII *RCs* are open, fluorescence intensity at 50 μs to maximum recorded fluorescence at *P*-step quotient

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cont. Tab. 1

Fluorescence parameter	Description
$F_v/F_m = 1 - F_o/F_m$	maximum quantum yield of primary PSII photochemistry
$F_v/F_o = \varphi_{Po}\ (1 - \varphi_{Po})$	efficiency of the water-splitting complex on the donor side of PSII
$V_K = (F_{0.3ms} - F_o)/(F_m - F_o)$	relative variable fluorescence at *K*-step (0.3 ms, *K*-band)
$V_J = (F_{2ms} - F_o)/(F_m - F_o)$	relative variable fluorescence at *J*-step (2 ms)
$dVG/dt_o = \Delta V_G/\Delta t_o$	slope of prompt fluorescence (*PF*) curve rise, between 50 and 100 μs
$dV/dt_o = 4\ (F_{0.3ms} - F_o)/(F_m - F_o)$	approximated initial slope of the fluorescent transient; this parameter is related to rate of closure of reaction centers
$S_m = A_m/(F_m - F_o)$	standardised area above the fluorescence curve between F_o and F_m is proportional to the pool size of the electron acceptors Q_A on the reducing side of PSII
S_S [RS] = V_J/M_o	normalised curve above O-J curve
S_S [VG] = Area to $F_J/(F_J - F_o)$	standardised area above the fluorescence curve between F_o and F_J
S_m/t_{Fm}	the normalised expression of *t* for F_m
$ABS/RC = M_o\ (1/V_J)\ [1 - (F_o/F_m)]$	apparent antenna size of active PSII *RC*
$DI_o/RC = (ABS/RC) - (TR_o/RC)$	dissipated energy flux per reaction center (*RC*) at *t* = 0
$ET_o/RC = M_o(1/V_J)\psi_0$	electron transport flux per reaction center (*RC*) at *t* = 0
$RE_o/RC = M_o(1/V_J)(1 - V_J)$	quantum yield of electron transport from Q_A^- to the PSI end electron acceptors
$psi_{(o)} = 1 - V_J$	probability (at time 0) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-
$RC/CS_o = \varphi_{Po}\ (V_J/M_o)\ F_o$	density of *RCs* (Q_A reducing PSII reaction centres)
DI_o/CS_o	dissipated energy flux per *CS*
TR_o/CS_o	trapping flux leading to Q_A reduction per *CS*
RE_o/CS_o	quantum yield of electron transport from Q_A^- to the PSI end electron acceptors
$PI_{abs} = \gamma_{RC/}(1 - \gamma_{RC})\ \varphi_{Po}/(1 - \varphi_{Po})\ \psi_o/(1 - \psi_o)$ $\gamma RC = Chl_{RC}/Chl_{total}$	performance index of PSII based to absorption
$PI_{total} = PI_{ABS}\ \delta_{Ro}/(1 - \delta_{Ro})$, where $\delta_{Ro} = (1 - V_J)/(1 - V_I)$	performance index, the performance of electron flux to the final PSI electron acceptors
DF_{total}	an indicator of the forces driving energy flows
$p2G = F_{50\mu s}\ C/(F_{2ms} - F_{50\mu s})$	overall grouping probability

Source: own elaboration based on: Strasser *et al.* [2004], Guidi and Degl'Innocenti [2011], Stirbet and Govindjee [2011], Brestic *et al.* [2012], and Kalaji *et al.* [2014].

To better visualise the influence of P deficiency stress on the dynamics of chlorophyll transients, the relative variable fluorescence was calculated. The next step was to calculate the differences in the relative variable fluorescence curves by subtracting the normalised fluorescence values (between the O and P levels) recorded in control plants and under stress. The relative variable fluorescence intensity (V_t) (Eq. 1) and the difference in relative variable fluorescence intensity (ΔV_t) (Eq. 2) were calculated:

$$V_t = \frac{F_t - F_o}{F_m - F_o} \quad (1)$$

$$\Delta V_{t,\text{stress}} = V_{t,\text{stress}} - V_{t,\text{control}} \quad (2)$$

STATISTICAL ANALYSIS

The basic statistical analyses of the rich data material conducted in the characteristic group-independent analyses showed mostly quite small and narrow differences. The research material therefore required a multidirectional approach. Chlorophyll fluorescence measurement method was used as one of the faster and non-invasive methods to assess plant condition and thus to detect stress response. Fluorimeters measure and calculate dozens of basic parameters from which additional parameters can subsequently be calculated. In this study, 62 parameters were determined from the OJIP indicator parameters. Some of them are different arithmetic approaches that give identical values in relation to other parameters; those that are repetitive were removed. Two pairs of parameters give different values, but their correlation with each other is functionally linear and gives a value of –1; these were left in the study because of the extended interpretation. The criteria for leaving a parameter for further study were its frequent occurrence in the literature and the second criterion a high coefficient of variation (*CV*). A total of 33 chlorophyll fluorescence parameters, represented by 348 cases, were used for statistical analyses. In order to describe the course of chlorophyll fluorescence values, their course was plotted in a graph. The differences between the chlorophyll fluorescence values of the control and phosphorus deficient systems were

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determined. The significance of the differences was determined for each measurement time point using a *t*-Student test. The experiment also examined the diversity of plant behaviour by measuring fluorescence on young leaves, old leaves, and fruits. The number of replicates for the control system and the phosphorus deficient system was the same. Young leaves and old leaves were measured with 6 replicates for each combination. Fruits were measured 24 times for each combination. All measurements were made on 5 dates. Date t_0 was the measurement on the day before the introduction of phosphorus deficiency stress. Each subsequent date from t_1 to t_4 was measured at 7-day intervals.

Selected parameters of chlorophyll-*a* fluorescence were subjected to statistical analyses. Pearson's correlation coefficients were determined for the data groups: control, phosphorus deficiency, leaf age. These analyses were performed in the Microsoft PowerBi Desktop package. Calculations of the space reduction described by 33 parameters, factor analysis was applied using the principal components method with the use of VariMax rotation. Calculations and graphs were made in the IBM SPSS Statistics ver. 28.

THYLAKOIDS ISOLATION, ABSORPTION SPECTROSCOPY, AND SDS POLYACRYLAMIDE GEL ELECTROPHORESIS

Thylakoid membranes were purified as previously reported without changes. Briefly, leaves were harvested, blended, and thylakoid membranes were isolated by centrifugation and subsequently solubilised with 20 mM n-Dodecyl-β-D-Maltoside (β-DDM) under strictly standardised conditions [Fey *et al.* 2008; Haniewicz *et al.* 2015; Piano *et al.* 2010]. After solubilisation, the supernatant was separated from the not-solubilised fraction by centrifugation (45000 × g, 10', 4°C). The solubilised fraction was used to analyse the protein composition of thylakoid membranes.

The protein content was calculated referring to the Chl *a* and Chl *b* concentrations from three independent measurements. The analysis was done photometrically in 80% (v/v) acetone and Chls concentrations were calculated according to Porra *et al.* [1989].

For denaturing Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE), 10% (w/v) separating polyacrylamide/urea gels with 4% (w/v) stacking gels were used [Piano *et al.* 2019]. Samples were denatured with Rotiload (Roth) at room temperature before loading, and after the electrophoretic separation, the gels were stained with Coomassie Brilliant Blue G250. Electrophoresis was carried out at 205 V for 5 h, at 4°C. All the tests were done in triplicates.

RESULTS

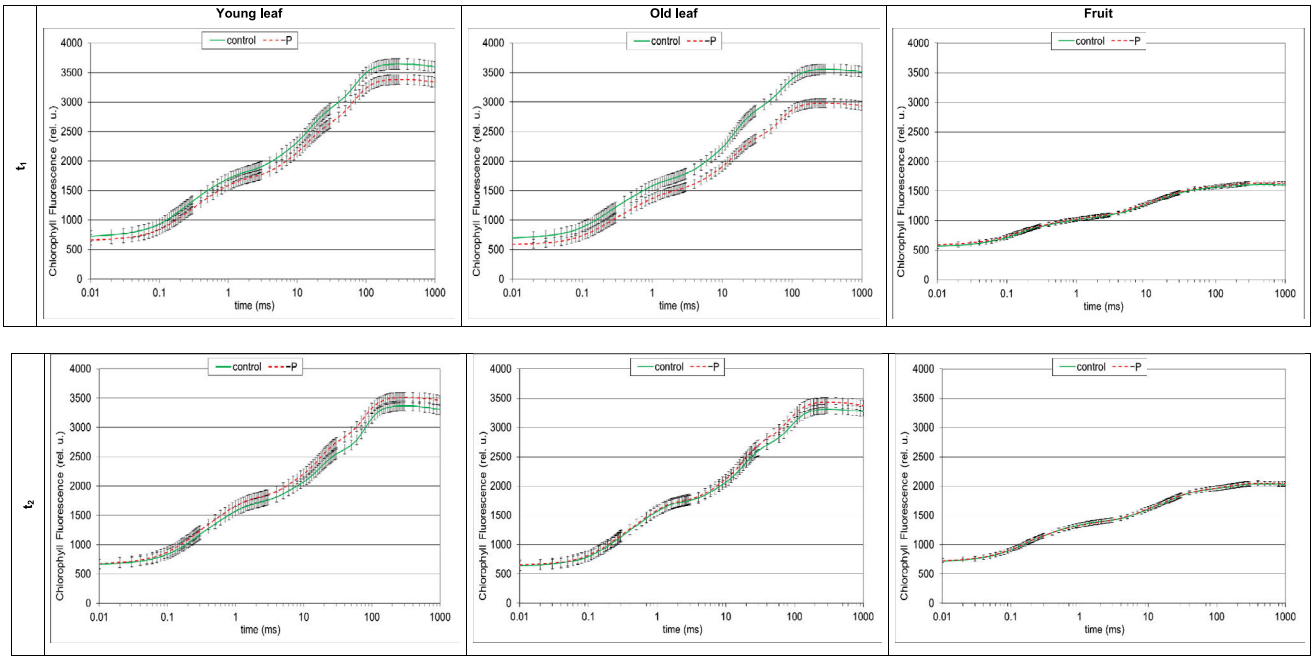
OJIP CURVES

From the shape of the OJIP curve, it can be concluded that the greatest changes occurred immediately after the introduction of the load (t_1). At the last time point, the course of the curve did not differ between the control plants and the plants treated with the deficiency. The changes observed were at the last two points of the curve: I and P. Note that these changes were greater in old leaves. The curves measured on the fruits had lower values than those measured on the leaves, but no changes were detected that could be due to P deficiency (Fig. 2).

ANALYSIS OF JIP TEST PARAMETER VALUES

A total of 33 parameters, characterised by high variability, were chosen for further study. These are: t for F_m; Area (A_m); Area to F_J; F_o; F_m; F_v; F_K = 0.3 ms; F_J = 2 ms; F_I = 30 ms; F_o/F_m; F_v/F_m; F_v/F_o; V_K; V_J; dV_G/dt_o; dV/dt_o; S_m; S_S [RS]; S_S [VG]; $S_m/t(F_m)$; *ABS/RC*; DI_o/RC; Et_o/RC; RE_o/RC; $psi_{(o)} = ET_o/TR_o$; RC/CS_o; DI_o/CS_o; TR_o/CS_o; RE_o/CS_o; PI_{abs}; PI_{total}; DF_{total}; *p2G*. The values of these parameters were normalised and shown in the radar plots (Fig. 3).

The mean values with standard error, standard deviation and coefficient of variation are shown in Table S1 (see



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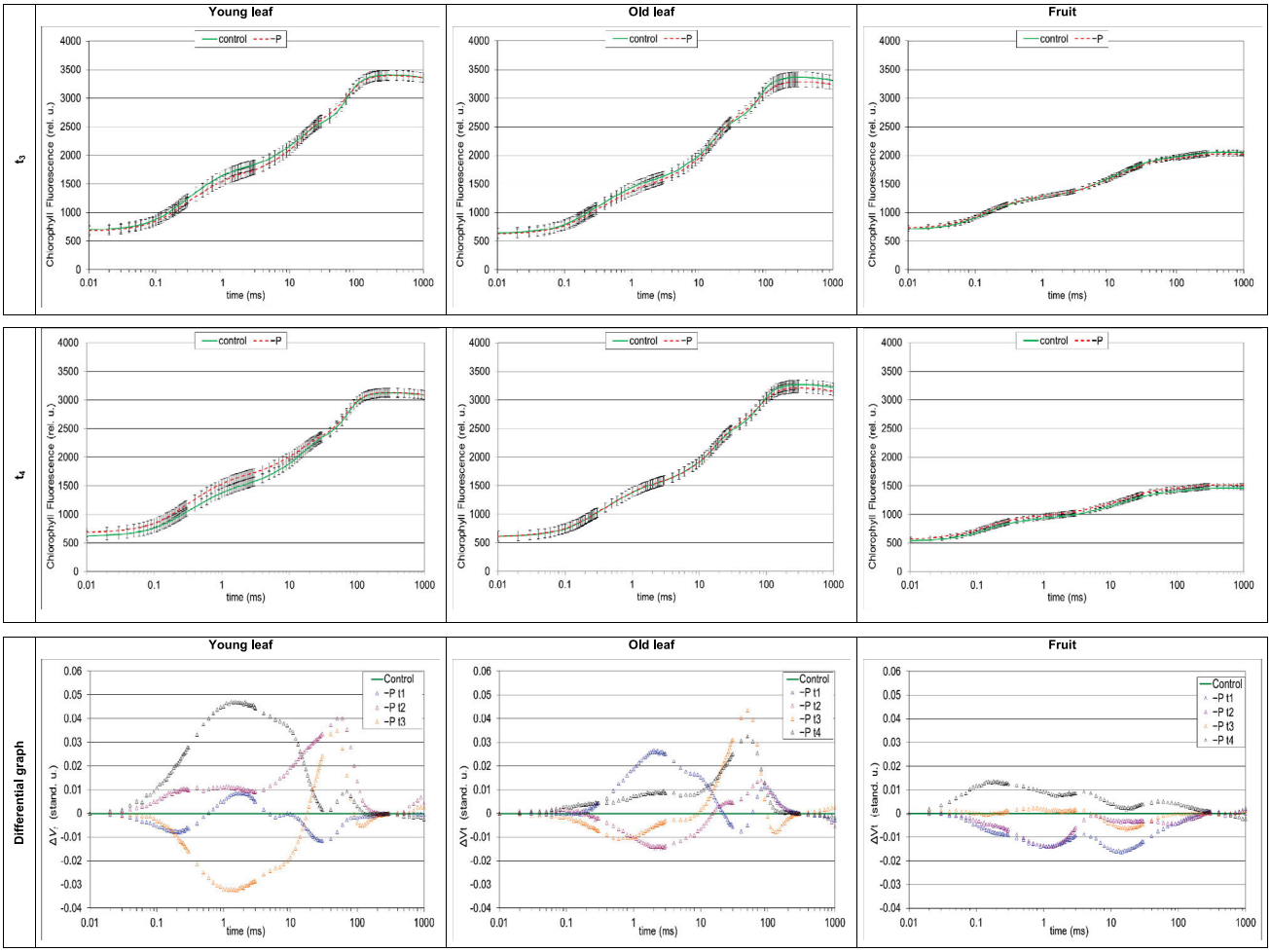

Fig. 2. Chlorophyll induction curves of cucumber measured on young and old leaves and fruits grown under conditions of phosphorus deficiency (–P) in four time periods (t_1–t_4); differential curves of ΔV_t (obtained by subtracting the control curve from the first sample); source: own study

supplementary material available at: https://www.jwld.pl/files/Supplementary-material-Sieczko.pdf). Tables S2–S5 show the values of the chosen coefficients (mean and standard error) for the control and phosphorus-deficient (–P) plants by leaf development level (young and old) and by the four measurement dates (t_1–t_4).

It was found that *p2G*, DF_{total} and PI_{total} were parameters that increased their values under phosphorus deficiency in the young leaf. In the old leaves, these were DF_{total} and PI_{total} parameters. It was also found that there were parameters whose value decreased under stress. RE_o/CS_o and TR_o/CS_o could belong to this group. At t_2 and t_3, no such changes were observed. At t_4, a decrease in the values of RE_o/CS_o, PI_{abs}, and PI_{total} parameters was observed, but only in the young leaf.

Figures S1 and S2 present the Pearson correlations between the individual JIP test parameters in non-stressed (control) and phosphorus-deficient plants. Stress did not cause major changes in the absolute value of the Pearson correlation coefficient. This conclusion suggests that classical statistical methods are not able to capture the subtle changes that occur in the readings of chlorophyll fluorescence values under stress. Therefore, other methods of describing the relationships, such as factor analysis, should be used.

Figure S3 shows the correlations between the coefficients without division into the use of a stress factor. Correlation values are mostly lower, which results from other values of the measured indicators in test plants and plants subjected to phosphorus deficiency stress. Due to the large variance, Pearson's *r* is much smaller.

The effect of phosphorus deficiency application on cucumber plant could be observed as early as day 7 (t_1) after its introduction. The greatest changes in indices compared to the control were observed in both young and old leaves. The experimental systems for the analysis of photosynthesis on leaves showed a high positive correlation with the PC1 component, which explained 61.56% of the total variation. The indices most correlated with component 1 (PC1) are indicated in bold in Table S6. Of the 33 indicators used for analysis, 26 are most strongly correlated with the PC1 component, of which 19 are positively correlated and seven are negatively correlated. The PC1 component determined the general condition of the plant as measured by chlorophyll fluorescence. Analysis of photosynthesis on fruit was strongly correlated with the PC1 component, but this relationship was negative. Indices such as F_o/F_m, V_J, V_K, *ABS/RC*, Dl_o/RC, Dl_o/CS_o, *t* for F_m best reflected the specificity of fruit photosynthesis. The results for the fruit on (Fig. 4) are in the left part of the *OXY* system, moreover, the distances between the results of the control and phosphorus deficient fruit are quite close, indicating small differences. Large differences between the control and deficient sites (connected by a dashed line in

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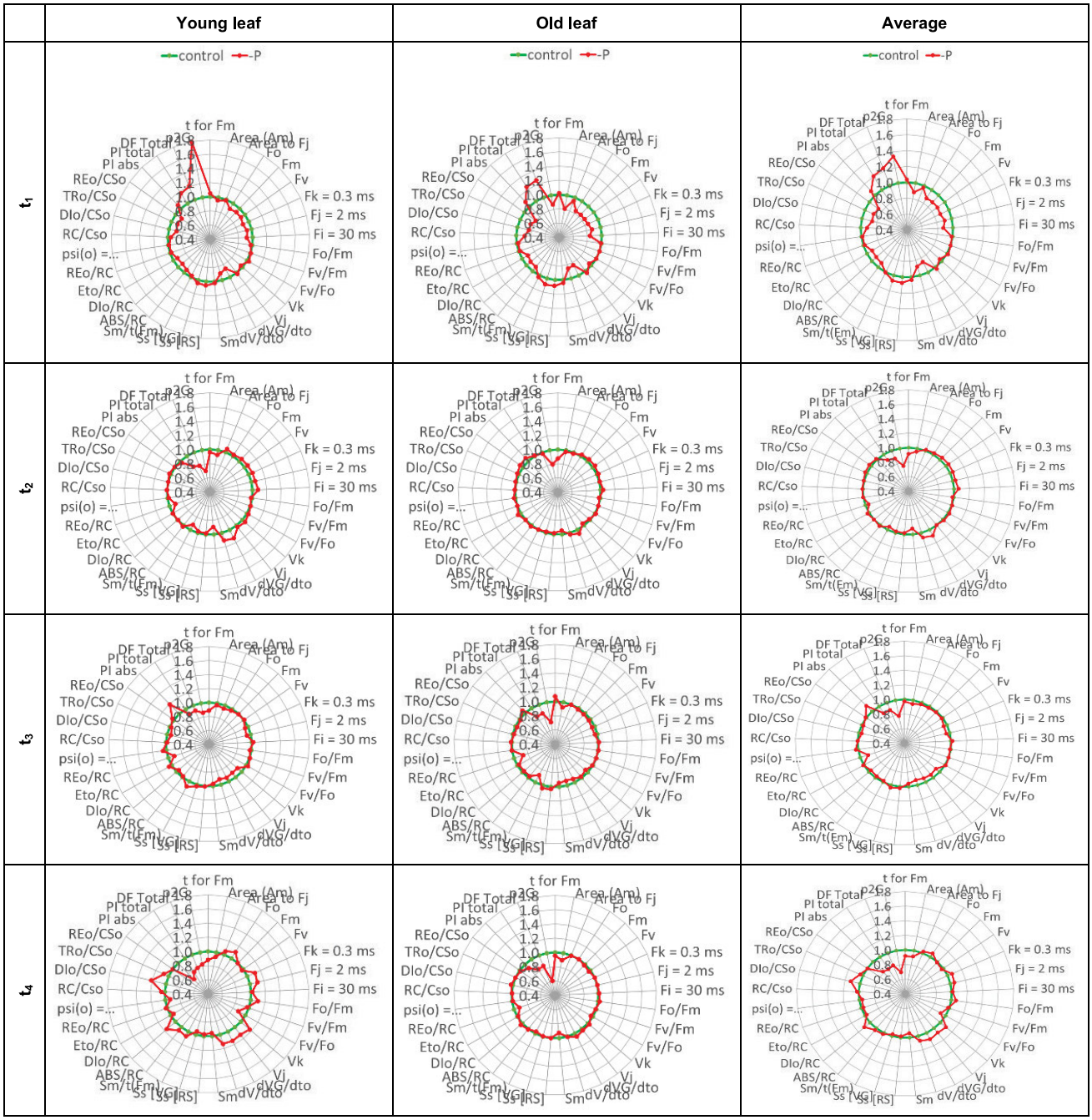

Fig. 3. JIP test parameters normalised to control plant values as radar plots for young leaves and older leaves at four measurement time points (t_1–t_4); standard error values of the mean are given in Tables S2–S4; source: own study

Figure 4 to facilitate identification) were observed for young and older leaves. These differences in the space of the first two components are most pronounced on the axis for PC2. At time t_1, PC2-related traits had the strongest difference between control and deficiency (Tab. S6, component 3 values in bold). The indicators positively associated with PC2 were: dVG/dt_o, F_K = 0.3 ms, F_o, dV/dt_o, TR_o/CS_o, F_J = 2 ms. The PC2 component determined the initial reaction rate of the plant and the change in the slope of the curve in the PSII process on the O-J section. The introduction of phosphorus deficiency stress as early as day 7 (t_1) of the observation leads to a decrease in these indices for the leaves. At later time points, PC1-related parameters showed greater differential significance. The PC1-related parameters were mainly general indices describing the condition of the plant.

Figure S4 shows the analysed components in the space of the two principal components (PC1 and PC3) explaining a total of 75.30% of the total variation with the experimental layouts.

ANALYSIS OF THE THYLAKOID PROTEIN PATTERN AND RELATED ABSORPTION PROPERTIES

Possible variations in protein representativity on the thylakoid samples were analysed by SDS-PAGE and absorption spectroscopy. The A subunit of photosystem I (PsaA), the major external

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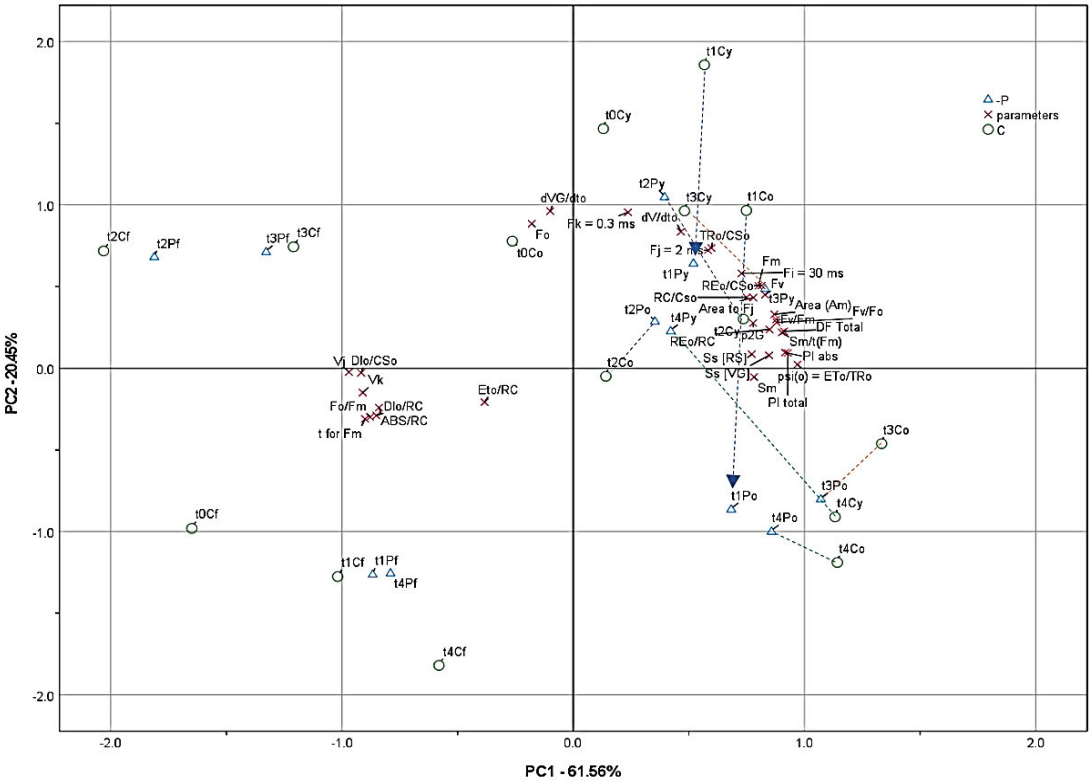
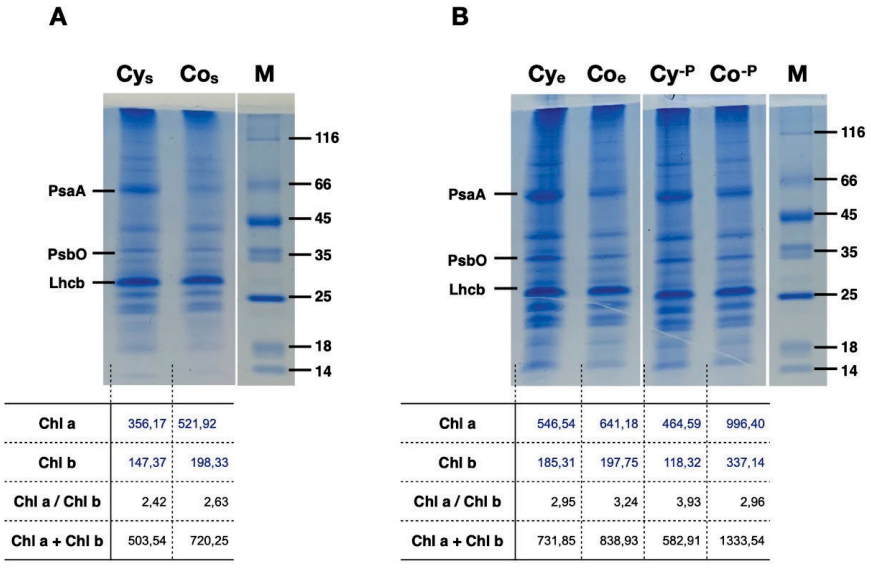


Fig. 4. The analysed components in the space of the first two principal components explaining a total of 82.12% of the overall variability together with the experimental layouts; C = control experimental sites, –P = phosphorus-deficient experimental sites), × = chlorophyll fluorescence parameters studied; experimental objects – the first two signs indicate the test date (t_0, t_1, t_2, t_3, t_4), the third sign indicates the test factor, control (C) or phosphorus deficiency (–P), the fourth sign indicates the part of the plant on which the measurement was made, young leaf (y), older leaf (o), fruit (f); the dashed lines connect the control objects with deficiencies at the same dates and on the same leaf stages; source: own study

antennas (light harvesting complex b – Lhcb), and the O subunit of photosystem II (PsbO) were used as an indirect discriminant for a qualitative correlation between controls and tested plants. Accordingly, before and after stress, we also measured the changes in protein pattern resolved by SDS-PAGE, and in absorption properties as ratio between Chl *a* (PsbA indicator) and Chl *b* (Lhcb indicator). This procedure allowed both a qualitative (SDS-PAGEs) and a (semi-) quantitative approach (absorption spectroscopy) – Figure 5. The effects were reflected on variations of PsaA, Lhcb, PsbO between young and old leaves of the same type (Cy_s vs Co_s, Cy_e vs Co_e, and Cy^{-P} vs Co^{-P}) (Fig. 5). More in details, the following reproducible differences could be observed: 1) decrease in PsbO and Lhcb in the young leaves hence an increase in the old; 2) increase the PsaA in the young leaves hence a decrease in the old. Therefore, with respect to phosphorous starvation, no major effect could be observed by these analyses (Fig. 5).



Chl a	356,17	521,92
Chl b	147,37	198,33
Chl a / Chl b	2,42	2,63
Chl a + Chl b	503,54	720,25

Chl a	546,54	641,18	464,59	996,40
Chl b	185,31	197,75	118,32	337,14
Chl a / Chl b	2,95	3,24	3,93	2,96
Chl a + Chl b	731,85	838,93	582,91	1333,54

Fig. 5. PAGEs of isolated thylakoids proteins from *Cucumis sativus* L.: A) samples from leaves harvested before to induce any kind of stress (controls, Cy_s = young and Co_s = old), B) samples from leaves harvested from control plants before to start the tests, and from controls and tested plants at the end of the starvation tests (controls, Cy_e = young and Co_e = old; phosphorous starvation, Cy^{-P} = young and Co^{-P} = old), M = the molecular marker for which the molecular weights expressed in kDa are indicated; PsaA = the A subunit of photosystem I, PsbO = the O subunit of photosystem II, Lcchb = light harvesting complex b; tables below each gel are reporting the chlorophyll concentration ($mg \cdot cm^{-3}$) per each analysed sample; source: own study

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DISCUSSION

The availability of nutrients during plant growth and development is crucial for the normal physiological state of the plant, including the maintenance of photosynthetic processes [Osman 2013; Smethurst *et al.* 2005]. In this study, we recorded *in vivo* chlorophyll-*a* fluorescence transients to analyse the changes in the light phase of photosynthesis in phosphorus-deficient cucumber plants.

Indeed, the deficiency of this nutrient led to changes in the shape of the chlorophyll-*a* fluorescence induction curve. The photosynthetic response of cucumber plants to nutrient deficiency was detected at different sites of the photosynthetic apparatus. In addition, it changed during the time they were exposed to the stress.

In our studies, we observed changes at the I and P points. A similar phenomenon was observed in barley plants by Frydenvang *et al.* [2015]. This confirms that the analysis of Chl *a* fluorescence induction curves can be used as a bioindicator to predict and monitor the nutrient status of plants. These changes were considered to reflect electron transfer through PSI and the development of a "jam" of electrons triggered by a blockade on the PSI acceptor side [Joly, Carpentier 2007; Schansker *et al.* 2005]. Considering the above findings, the disappearance of the *I*-step in plants under P deficiency may be related to the perturbations in ETC beyond PSII and/or the reduction of PSI-acceptors (inactivation of PSI and suppression of cyclic phosphorylation) [Frydenvang *et al.* 2015; Joly *et al.* 2010]. Moreover, several studies [Hamdani *et al.* 2015] have shown a clear relationship between the slope of IP phase and photosynthetic performance and even plant morphology.

Recording OJIP fluorescence transients in our experiments, followed by analysis with the JIP test, allowed quantification of photosynthetic parameters that provide insight into phosphorus deficiency-induced changes in PSII function. We detected significant differences in some parameters in cucumber plants under this stress. Parameters that decreased during the stress were: $p2G$, PI_{abs}, PI_{total}, RE_o/CS_o, and TR_o/CS_o. The results of Lin *et al.* [2009], that P deficiency decreased total electron carriers per *RC* (EC_o/RC), yields (TR_o/ABS, F_v/F_m, ET_o/TR_o, RE_o/ET_o, ET_o/ABS and RE_o/ABS), fluxes (RE_o/RC and RE_o/CS_o) and fractional reduction of PSI end electron acceptors, and damaged all photochemical and non-photochemical redox reactions as indicated by decreases in PI_{abs} and PI_{total}. This implies that plants under P deficiency stress have a decreased capacity of electron transport. This means that ATP synthesis and RuBP regeneration are blocked. Moreover, the application of PCA separated a large number of these parameters according to their influence on the stress response of plants. Some groups of parameters were found to be sensitive to P deficiency and could be used as stress markers. The works by Samborska *et al.* [2019] and Horaczek *et al.* [2020] present similar parameters as identifiers of abiotic stresses in plants.

We conclude that our results discussed above could be used in the discovery of sensitive bioindicators of phosphorus deficiency in cucumber plants. Since most JIP test parameters are linked to mathematical equations, the application of advanced statistical tools, such as principal component analysis, should be considered very useful for stress identification [Goltsev *et al.* 2012; Kalaji *et al.* 2014; 2017b]. Our research has shown that changes in the shape of the chlorophyll-*a* fluorescence induction curve in the case of some stressors can serve as a tool for rapid and/or preliminary diagnosis in the field. Changes in the I and P points of the induction curve can be used as a specific physiological bioindicator for early detection of phosphorus deficiency in cucumber plants. Analysis of the thylakoid protein pattern and related absorption characteristics showed no significant effects of phosphorus deficiency. This confirms that chlorophyll-*a* fluorescence is a very sensitive method for such studies.

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